

# Rapid detection of tetracycline resistance in bovine *Pasteurella multocida* isolates by MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA)

## SCIENTIFIC REPORTS

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### Rapid detection of tetracycline resistance in bovine *Pasteurella multocida* isolates by MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA)

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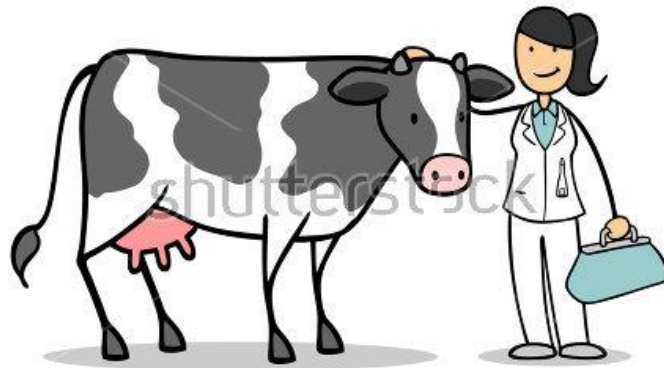
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# INTRODUCTION

- Infectious bronchopneumonia: leading cause antimicrobial use in calves
- ↗ Pressure intensive antimicrobial consumption food animals

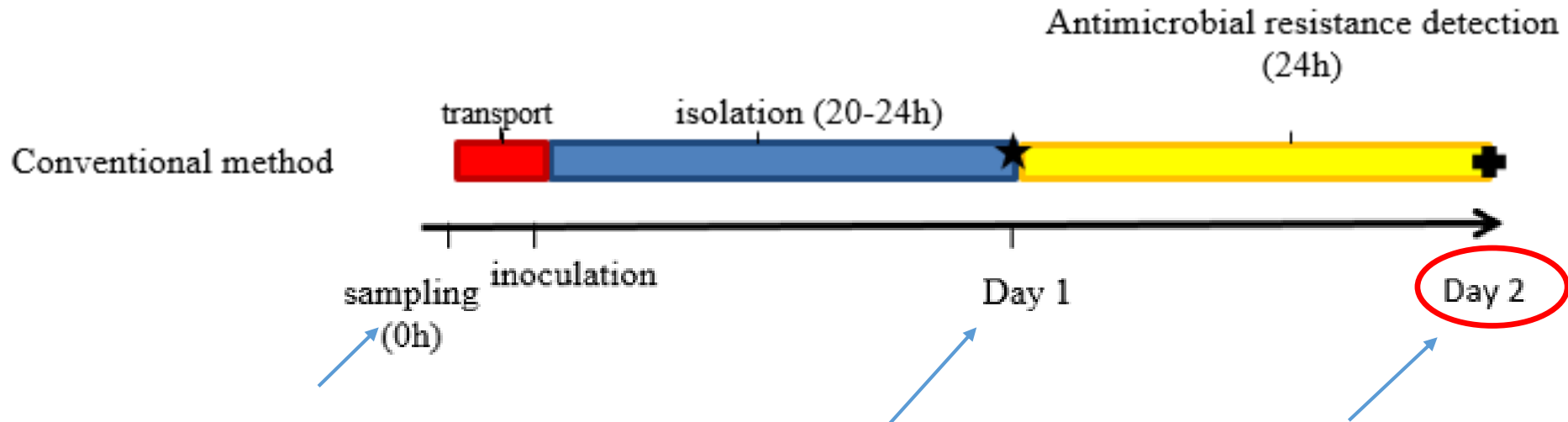


# INTRODUCTION

- Reduction and rational antimicrobial use  
=> TOP PRIORITY!
- Formularies
  - 1<sup>st</sup> choice products
  - Certain classes: identification and susceptibility test requested



# INTRODUCTION



Broncho-  
alveolar lavage



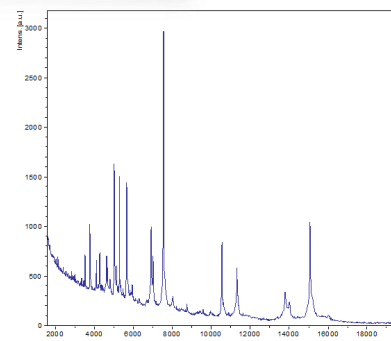
Identification



Disk diffusion

=>minimum 2 days!

# MALDI-TOF MS: Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry



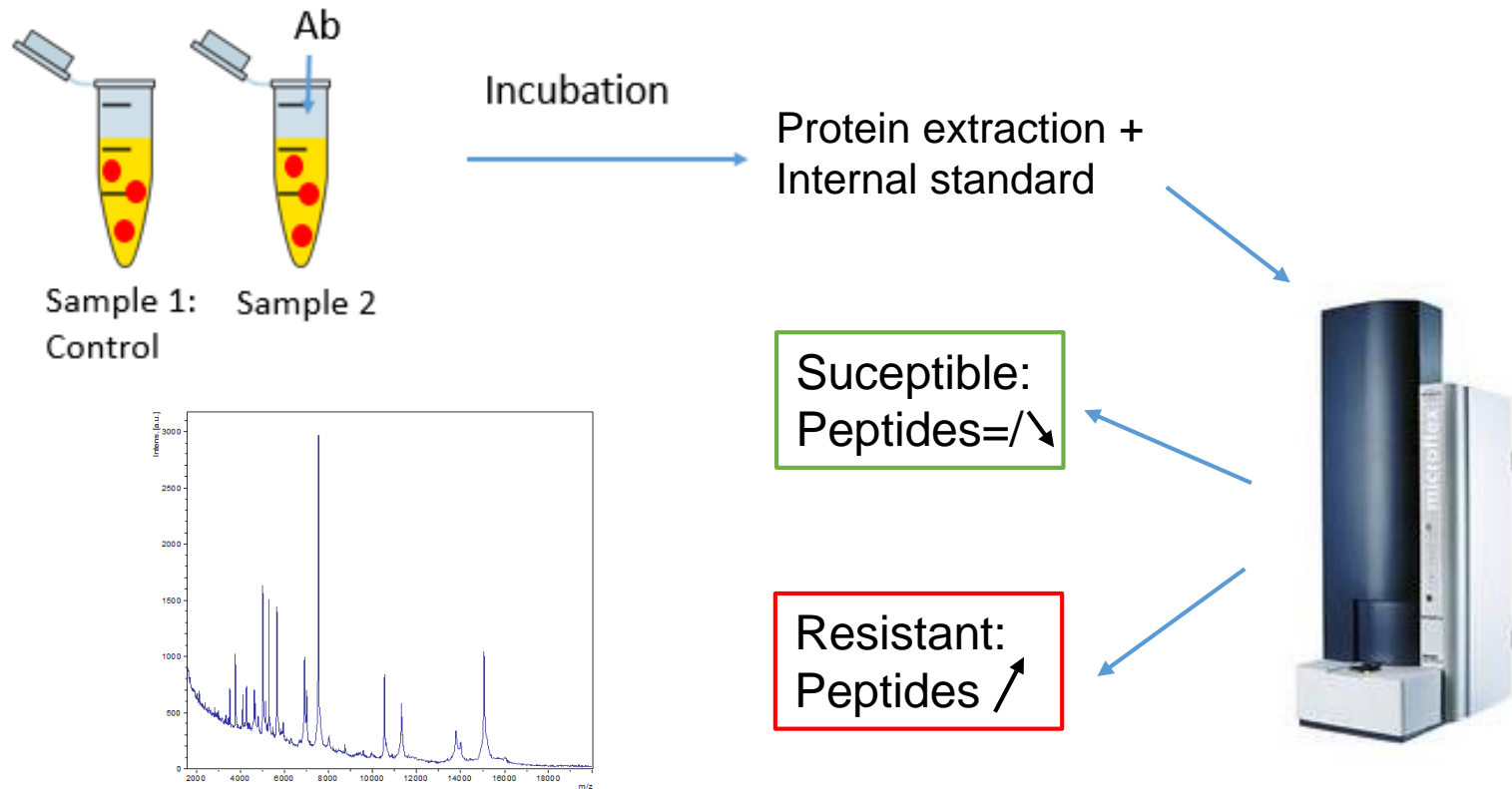
'fingerprint'

# OBJECTIVE

- **Design and validate MBT-ASTRA** for ***Pasteurella multocida*** (ubiquitous respiratory pathogen in cattle) and **tetracycline** (frequently used antimicrobial)
- Determine **diagnostic accuracy** of **MBT-ASTRA** and **disk diffusion** with MIC-gradient strip test



- MBT-ASTRA: Maldi Biotyper-antibiotic susceptibility test rapid assay  
=> semi-quantitative MALDI-TOF MS to analyse susceptibility/ resistance status of bacteria in few hours

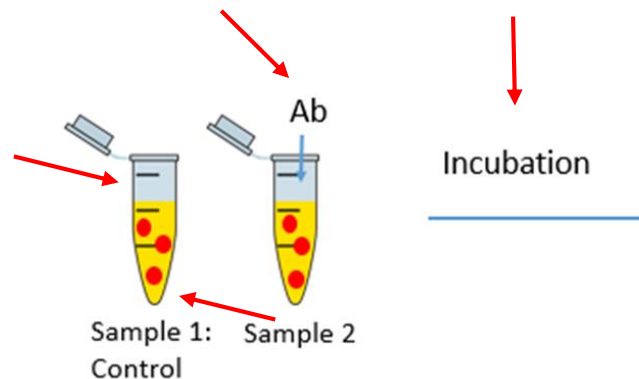


# DESIGN MBT-ASTRA

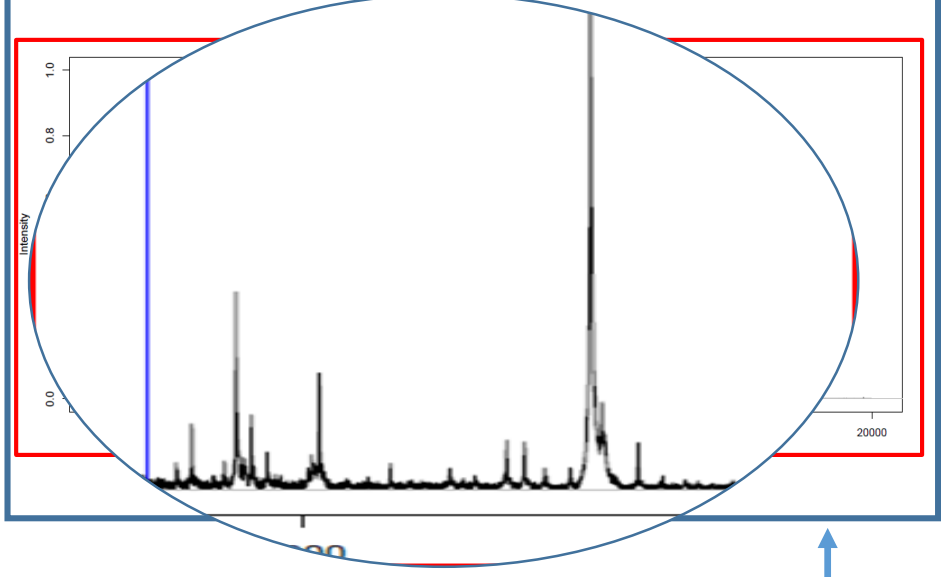
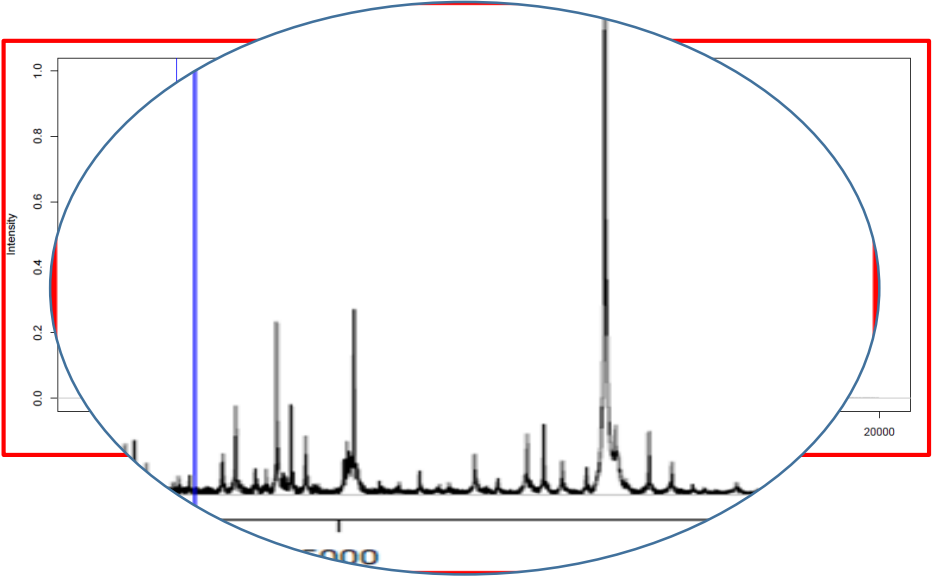
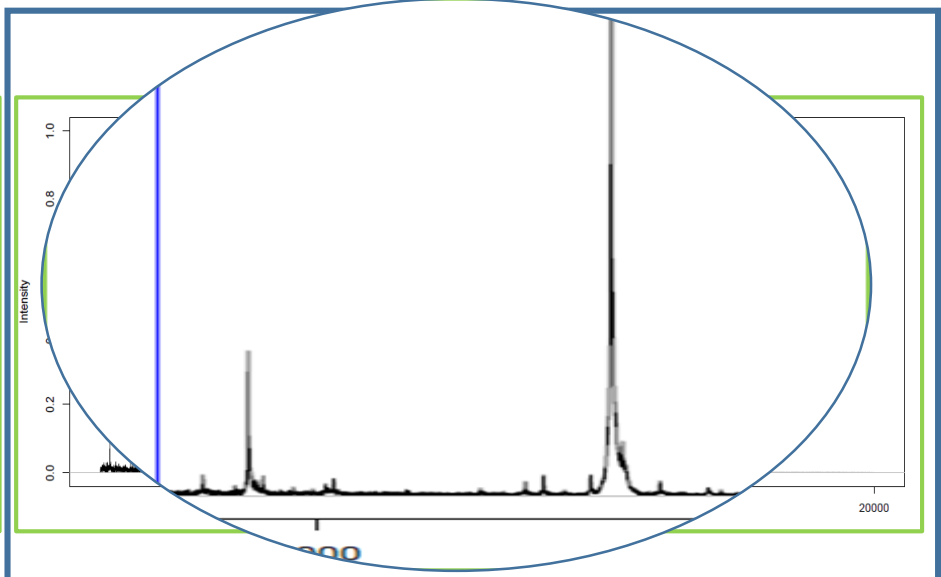
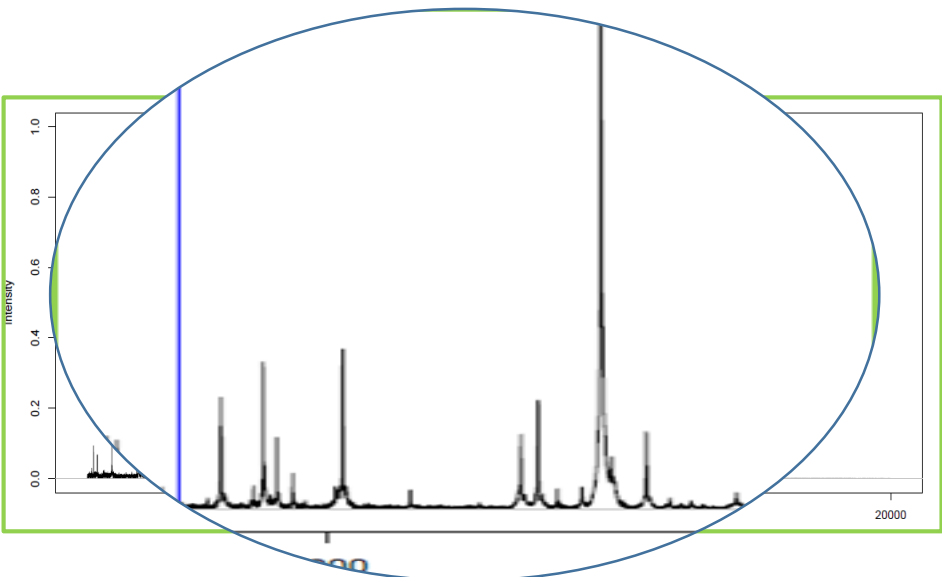


Conditions to determine:

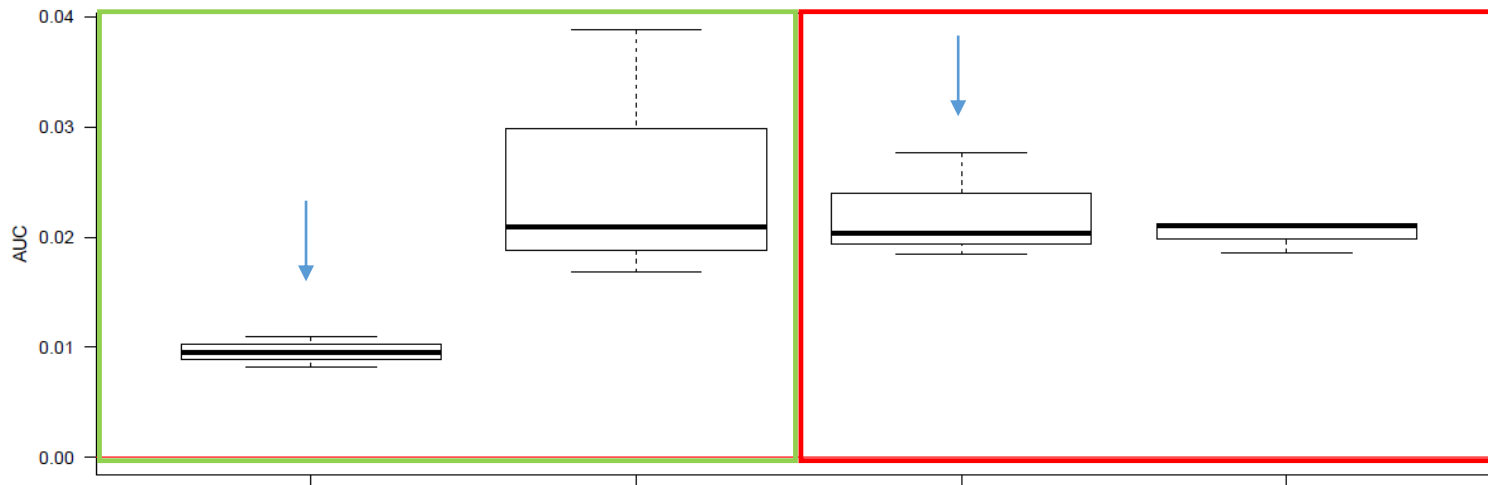
1. Growth medium: **Cation-adjusted Mueller Hinton Broth**
2. Starting concentration of *P. multocida*:  $10^7$  cfu/mL
3. Concentration of tetracycline:  $4\mu\text{g/mL}$
4. Incubation time: **3 hours!!**





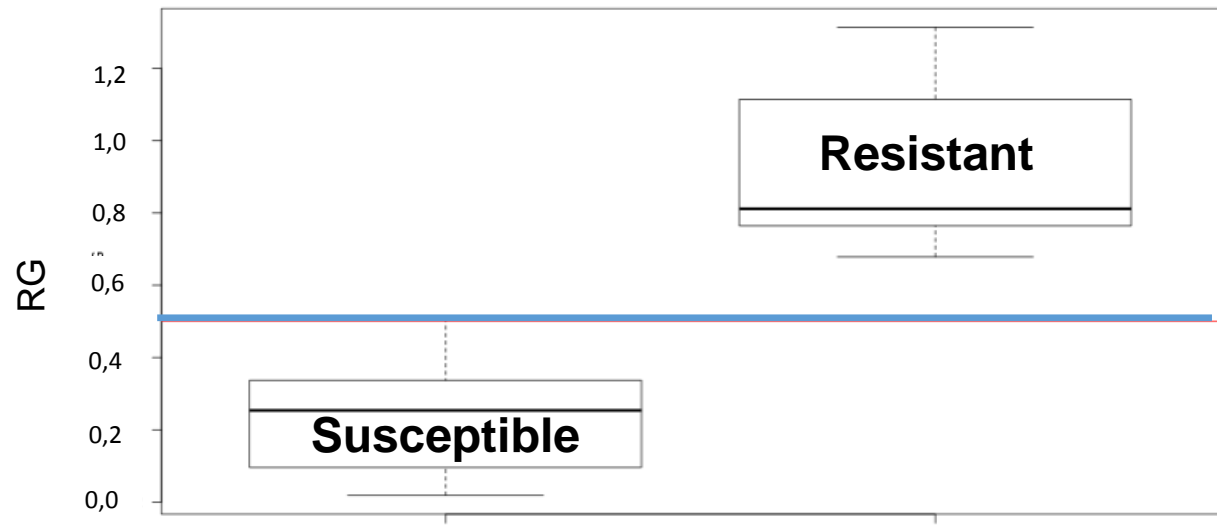


## Area under the curve (AUC)



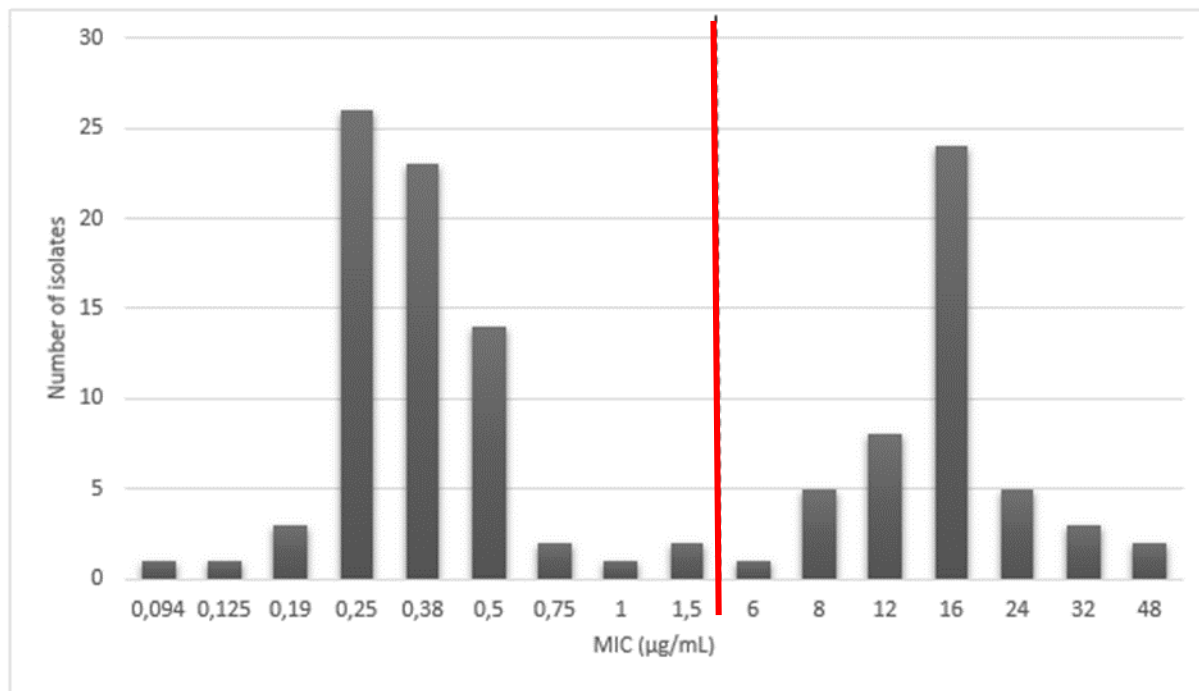
## Relative growth ratio (RG)

$$\text{RG: } \frac{\text{AUC} + \text{Ab}}{\text{AUC} - \text{Ab}}$$

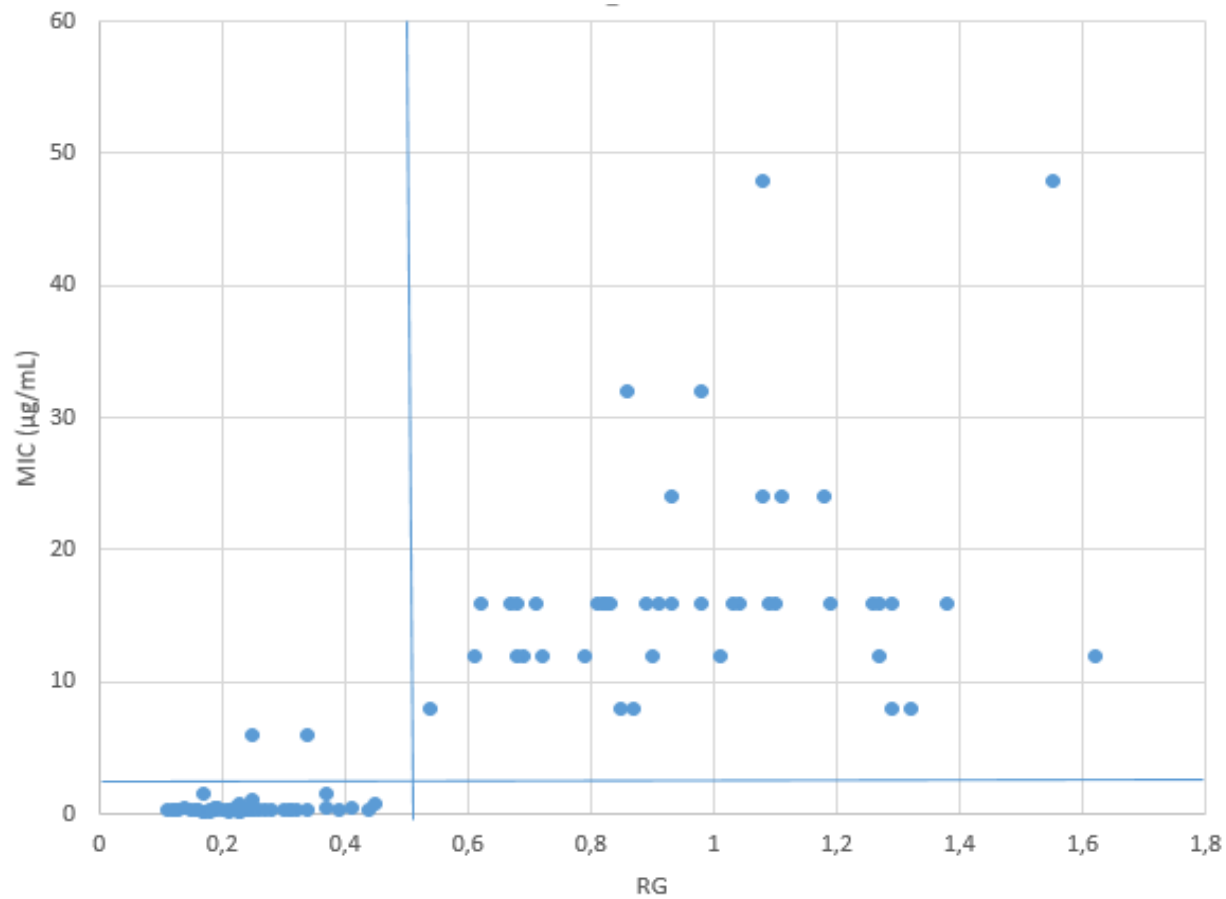


# DIAGNOSTIC ACCURACY MBT-ASTRA

- Database 100 recent clinical strains of *P. multocida* with MIC- values for tetracycline by MIC-gradient strip test



# DIAGNOSTIC ACCURACY MBT-ASTRA



# DIAGNOSTIC ACCURACY MBT-ASTRA AND DISK DIFFUSION

Test		Reference test (MIC-gradient strip test)	
		<i>Resistant</i>	<i>Susceptible</i>
MBT-ASTRA	<i>Resistant</i>	95.7% (44/46)	0% (0/54)
	<i>Susceptible</i>	4.3% (2/46)	100% (54/54)
	<i>Total</i>	100% (46/46)	100% (54/54)
Disk diffusion	<i>Resistant</i>	93.5% (43/46)	3.7% (2/54)
	<i>Susceptible</i>	6.5% (3/46)	96.2% (52/54)
	<i>Total</i>	100% (46/46)	100% (54/54)

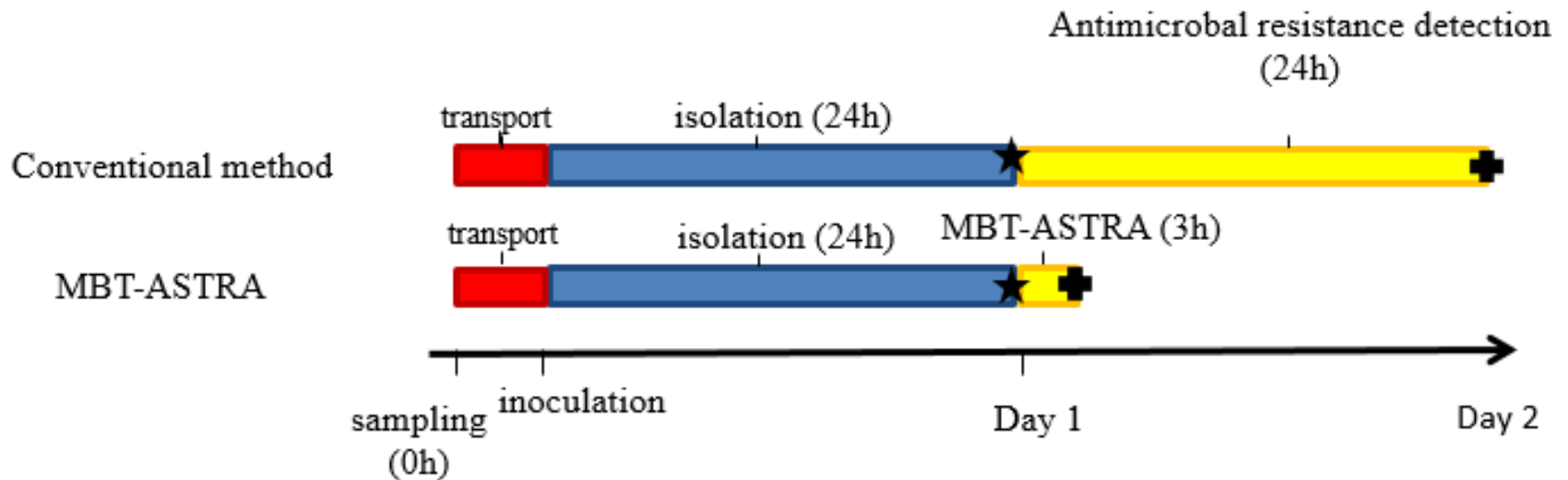


# DIAGNOSTIC ACCURACY MBT-ASTRA AND DISK DIFFUSION



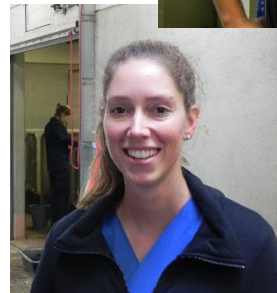
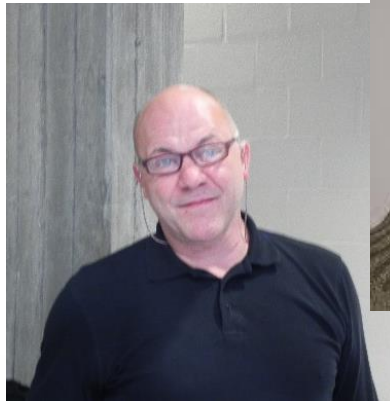
	Disk diffusion	MBT-ASTRA
<i>Essential agreement</i>	95%	98%
<i>Very major error</i>	3%	2%
<i>Major error</i>	2%	0%
<i>Minor error</i>	0%	0%
<i>Sensitivity</i>	93.5%	95.7%
	(86.3%, 100.6%)	(89.8%, 101.5%)
<i>Specificity</i>	96.3%	100%
	(91.3%, 101.3%)	(100.0%, 100.0%)
<i>RPV</i>	95.6%	100%
	(89.5%, 101.6%)	(100.0%, 100.0%)
<i>SPV</i>	94.5%	96.4%
	(88.5%, 100.5%)	(91.6%, 101.3%)

# CONCLUSION



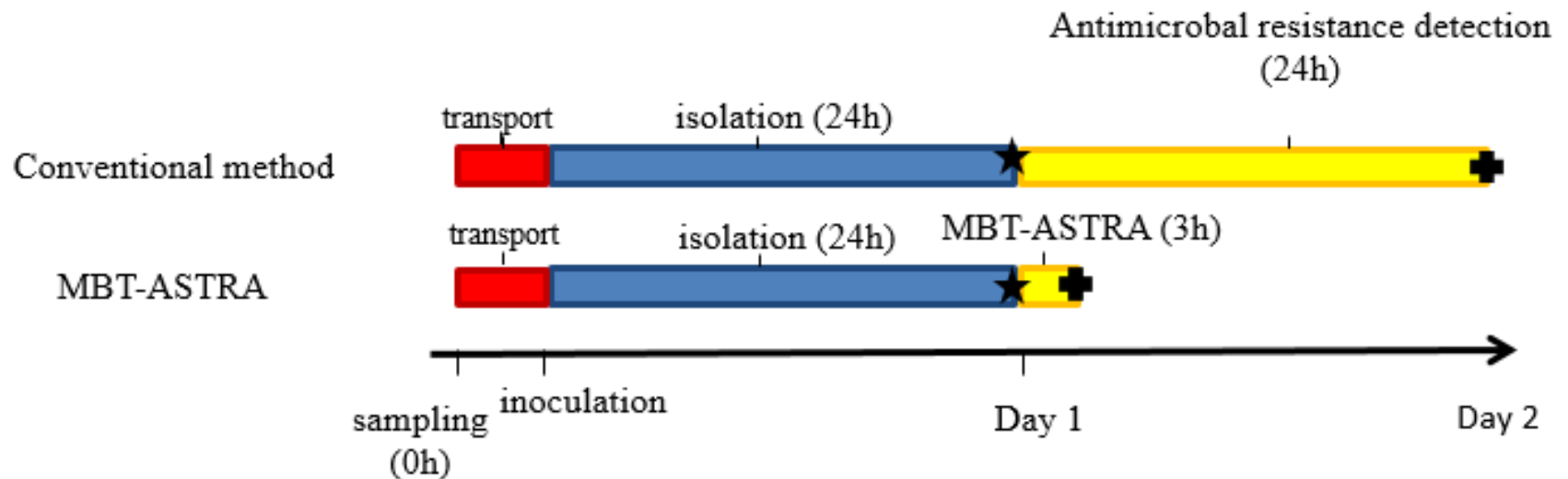
**Identification and susceptibility testing possible  
in the same day!!**

# ACKNOWLEDGEMENTS





# CONCLUSION



<https://www.nature.com/articles/s41598-018-31562-8>